CLAIMS

1. A method for isolating a polynucleotide of interest that is present in the genome of a mycobacterium strain and/or is expressed by said mycobacterium strain and that is absent or altered in the genome of a different mycobacterium strain and/or is not expressed in said different mycobacterium strain, said method comprising the use of at least one clone belonging to a genomic DNA library of a given mycobaterium strain, said DNA library being cloned in a bacterial artificial chromosome (BAC) vector.

2. The method according to claim 1, wherein the BAC-based DNA library has beeen constructed from genomic DNA of *Mycobacterium tuberculosis*.

3. The method according to claim 2, wherein the BAC-based DNA library has beeen constructed from genomic DNA of *Mycobacterium tuberculosis* strain H37Rv.

4. The method according to claim 3, wherein the BAC-based DNA library has been deposited in the Collection Nationale de Cultures de Microorganismes (CNCM) on November 19, 1997 under the accession number I-1945.

5. The method according to claim 1, wherein the BAC-based DNA library has been constructed from genomic DNA of *Mycobacterium bovis*.

6. The method according to claim 5, wherein the BAC-based DNA library has been constructed from the genomic DNA of *Mycobacterium bovis* BCG strain Pasteur.

7. The method according to claim 6, wherein said DNA library contains approximatively 1600 clones and wherein the genomic DNA is cloned into a recombinant pBeloBAC11 vector with an average insert size of approximately 80 kb.

8. The method according to claim 6 or 7, wherein the at least one BAC-based DNA library has been deposited in the Collection Nationale de Cultures de Microorganismes (CNCM) on June 30, 1998 under the accession number I-2049.

9. A method of isolating a polynucleotide of interest that is present in a genome of a first mycobacterium strain or that is expressed by the first mycobacterium strain and that is absent or altered in a genome of a second mycobacterium strain or that is not expressed by the second mycobacterium strain, said method comprising :

a) providing at least one polynucleotide contained in a clone of a bacterial artificial chromosome (BAC) DNA library of the first mycobacterium strain;

b) providing at least one genomic or cDNA polynucleotide from a second mycobacterium strain that is different from the first mycobacterium strain or at least one polynucleotide contained in a clone of a BAC DNA library prepared from the genome of the second mycobacterium strain;

c) contacting under hybridizing conditions the polynucleotide of step a) with the polynucleotide of step b); and

d) isolating the polynucleotide of step a) that has not formed a hybrid complex with the polynucleotide of step b).

10. The method of claim 9, wherein the polynucleotide contained in a clone of a BAC DNA library of the first or second mycobacterium strain is prepared by the following procedure :

1) digesting at least one recombinant BAC clone by an appropriate restriction endonuclease to yield a polynucleotide insert of interest; and

2) isolating the polynucleotide insert of interest.

11. A purified polynucleotide of interest that has been isolated according to the method of claim 9.

12. The purified polynucleotide of claim 11 which contains at least one Open Reading Frame (ORF).

13. The purified polynucleotide of claim 12, which is SEQ ID N0:1.

14. The purified polynucleotide of claim 12, wherein said polynucleotide is selected from the group consisting of:

a) a polynucleotide comprising at least 8 consecutive nucleotides of SEQ ID N0:1;

b) a polynucleotide having a sequence fully complementary to SEQ ID N0:1; and

c) a polynucleotide that hybridizes under stringent hybridization conditions with the polynucleotide defined in a) or with the polynucleotide defined in b).

15. The purified polynucleotide of claim 14, which is SEQ ID N0:2.

16. The purified polynucleotide of claim 14, which is SEQ ID N0:3.

17. The purified polynucleotide of claim 12, wherein the ORF encodes all or part of a polypeptide involved in the pathogenicity of a mycobacterium strain.

18. The purified polynucleotide of claim 12, wherein the ORF encodes all or part of a Polymorphism Glycine Rich Sequence (PGRS).

19. The purified polynucleotide of claim 18, which is SEQ ID N0:4.

20. The purified polynucleotide of claim 18, which is selected from the group consisting of:

a) a polynucleotide comprising at least 8 consecutive nucleotides the of SEQ ID N0:5 ;

b) a polynucleotide having a sequence that is fully complementary to SEQ ID N0:5 ;

c) a polynucleotide that hybridizes under stringent hybridization conditions with the polynucleotide defined in a) or with the polynucleotide defined in b).

21. A pair of the purified polynucleotides as claimed in claim 11.

22. A *Mycobacterium tuberculosis* strain Rv37 genomic DNA library that has been deposited in the Collection Nationale de Cultures de Microorganismes under accession number I-1945, wherein said genomic DNA library comprises recombinant bacterial artificial chromosome vectors.

23. A recombinant bacterial artificial chromosome (BAC) vector, which belongs to the genomic DNA library of claim 22.

24. The recombinant BAC vector of claim 23, which is selected from the group consisting of :

Rv101; Rv102; Rv103; Rv104; Rv105; Rv106; Rv107; Rv108; Rv109; Rv10; Rv110; Rv111; Rv112; Rv113; Rv114; Rv115; Rv116; Rv117; Rv118; Rv119; Rv11; Rv120; Rv121; Rv122; Rv123; Rv124; Rv126; Rv127; Rv128; Rv129; Rv130; Rv132; Rv134; Rv135; Rv136; Rv137; Rv138; Rv139; Rv13; Rv140; Rv141; Rv142; Rv143; Rv144; Rv145; Rv146; Rv147; Rv148; Rv149; Rv14; Rv150; Rv151; Rv152; Rv153; Rv154; Rv155; Rv156; Rv157; Rv159; Rv15; Rv160; Rv161; Rv162; Rv163; Rv164; Rv165; Rv166; Rv167; Rv169; Rv16; Rv170; Rv171; Rv172; Rv173; Rv174; Rv175; Rv176; Rv177; Rv178; Rv179; Rv17; Rv180; Rv181; Rv182; Rv183; Rv184; Rv185; Rv186; Rv187; Rv188; Rv18; Rv190; Rv191; Rv192; Rv193; Rv194; Rv195; Rv196; Rv19; Rv1; Rv201; Rv204; Rv205; Rv207; Rv209; Rv20; Rv214; Rv215; Rv217; Rv218; Rv219; Rv21; Rv220; Rv221; Rv222; Rv223; Rv224; Rv225; Rv226; Rv227; Rv228; Rv229; Rv22; Rv230; Rv231; Rv232; Rv233; Rv234; Rv235; Rv237; Rv240; Rv241; Rv243; Rv244; Rv245; Rv246; Rv247; Rv249; Rv24; Rv251; Rv252; Rv253; Rv254; Rv255; Rv257; Rv258; Rv259; Rv25; Rv260; Rv261; Rv262; Rv263; Rv264; Rv265; Rv266; Rv267; Rv268; Rv269; Rv26; Rv270; Rv271; Rv272; Rv273; Rv274; Rv275; Rv276; Rv277; Rv278; Rv279; Rv27; Rv280; Rv281; Rv282; Rv283; Rv284; Rv285; Rv286; Rv287; Rv288; Rv289; Rv28; Rv290; Rv291; Rv292; Rv293; Rv294; Rv295; Rv296; Rv29; Rv2; Rv301; Rv302; Rv303; Rv304; Rv306; Rv307; Rv308; Rv309; Rv30; Rv310; Rv311; Rv312; Rv313; Rv314; Rv315; Rv316; Rv317; Rv318; Rv319; Rv31; Rv32; Rv322; Rv327; Rv328; Rv329; Rv32; Rv330; Rv331; Rv333; Rv334; Rv335; Rv336; Rv337; Rv338; Rv339; Rv33; Rv340; Rv341; Rv343; Rv344; Rv346; Rv347; Rv348; Rv349; Rv34; Rv350; Rv351; Rv352; Rv353; Rv354; Rv355; Rv356; Rv357; Rv358; Rv359; Rv35; Rv360; Rv361; Rv363; Rv364; Rv365; Rv366; Rv367; Rv368; Rv369; Rv36; Rv370; Rv371; Rv373; Rv374; Rv375; Rv376; Rv377; Rv378; Rv379; Rv37; Rv381; Rv382; Rv383; Rv384; Rv385; Rv386; Rv387; Rv388; Rv389; Rv38; Rv390; Rv391; Rv392; Rv393; Rv396; Rv39; Rv3; Rv40; Rv412; Rv413; Rv414; Rv415; Rv416; Rv417; Rv418; Rv419;

Rv41; Rv42; Rv43; Rv44; Rv45; Rv46; Rv47; Rv48; Rv49; Rv4; Rv50; Rv51; Rv52; Rv53; Rv54; Rv55; Rv56; Rv57; Rv58; Rv59; Rv5; Rv60; Rv61; Rv62; Rv63; Rv64; Rv65; Rv66; Rv67; Rv68; Rv69; Rv6; Rv70; Rv71; Rv72; Rv73; Rv74; Rv75; Rv76; Rv77; Rv78; Rv79; Rv7; Rv80; Rv81; Rv82; Rv83; Rv84; Rv85; Rv86; Rv87; Rv88; Rv89; Rv8; Rv90; Rv91; Rv92; Rv94; Rv95; Rv96 and Rv9.

25. The recombinant BAC vector of claim 23, which is selected from the group consisting of:

Rv234; Rv351; Rv166; Rv35; Rv415; Rv404; Rv209; Rv272; Rv30; Rv228; Rv233; Rb38; Rv280; Rv177; Rv48; Rv374; Rv151; Rv238; Rv156; Rv92; Rv3; Rv403; Rv322; Rv243; Rv330; Rv285; Rv233; Rv219; Rv416; Rv67; Rv222; Rv149; Rv279; Rv87; Rv273; Rv266; Rv25; Rv136; Rv414; Rv13; Rv289; Rv60; Rv104; Rv5; Rv165; Rv215; Rv329; Rv240; Rv19; Rv74; Rv411; Rv167; Rv56; Rv80; Rv164; Rv59; Rv313; Rv265; Rv308; Rv220; Rv258; Rv339; Rv121; Rv419; Rv418; Rv45; Rv217; Rv134; Rv17; Rv103; Rv21; Rv22; Rv2; Rv270; Rv267; Rv174; Rv257; Rv44; Rv71; Rv7; Rv27; Rv191; Rv230; Rv128; Rv407; Rv106; Rv39; Rv255; Rv74; Rv355; Rv268; Rv58; Rv173; Rv264; Rv417; Rv401; Rv144; Rv302; Rv81; Rv163; Rv281; Rv221; Rv420; Rv175; Rv86; Rv412; Rv73; Rv269; Rv214; Rv287; Rv42 and Rv143.

26. A *Mycobacterium bovis* BCG strain Pasteur genomic DNA library, wherein said genomic DNA library comprises recombinant bacterial artificial chromosome vectors.

27. A *Mycobacterium bovis* BCG strain Pasteur genomic DNA library according to claim 26, wherein said DNA library contains approximatively 1600 clones and wherein the genomic DNA is cloned into a recombinant pBeloBAC11 vector with an average insert size of approximately 80 kb.

28. A *Mycobacterium bovis* BCG strain Pasteur genomic DNA library according to claim 26, that has been deposited in the Collection Nationale de

Cultures de Microorganismes (CNCM) on June 30, 1998 under the accession number I-2049.

29. A recombinant bacterial artificial chromosome (BAC) vector, which belongs to the genomic DNA library of claims 26 to 28.

30. A recombinant BAC vector according to claim 29, which is selected from the group consisting of:
X0001; X0002; X0003; X0004; X0006; X0007; X0008; X0009; X0010; X0012; X0013; X0014; X0015; X0016; X0017; X0018; X0019; X0020; X0021 and X0175.

31. A method for detecting a mycobacterial nucleic acid in a biological sample comprising the steps of:

a) contacting the recombinant BAC vector according to claim 23 or 29, or a purified polynucleotide according to claim 11 with the mycobacterial nucleic acid in the biological sample ; and

b) detecting a hybrid nucleic acid molecule formed between said recombinant BAC vector or said purified polynucleotide and the mycobacterial nucleic acid in the biological sample.

32. The method of claim 31, further comprising before step a), making the mycobacterial nucleic acid in the biological sample available to a hybridization reaction.

33. A method for detecting mycobacterial nucleic acid in a biological sample comprising the steps of:

a) contacting a first polynucleotide according to claim 11 that has been immobilized onto a substrate with the mycobacterial nucleic acid in the biological sample ; and

b) contacting a hybrid nucleic acid molecule formed between said first polynucleotide and the mycobacterial nucleic acid in the biological sample with a second, labeled polynucleotide according to claim 11, wherein said

second polynucleotide and said first polynucleotide have non-overlapping sequences.

34. The method of claim 33, further comprising before step a), making the mycobacterial nucleic acid in the biological sample available to a hybridization reaction.

35. The method of claim 33 or 34, further comprising before step b), removing the mycobacterial nucleic acid that is not hybridized with the immobilized first polynucleotide.

36. A method for detecting mycobacterial nucleic acid in a biological sample comprising the steps of:

a) contacting the mycobacterial nucleic acid in the biological sample with a pair of purified polynucleotides according to claim 21 ;

b) amplifying said mycobacterial nucleic acid ; and

c) detecting the amplified mycobacterial nucleic acid.

37. The method of claim 36, further comprising before step a), making the mycobacterial nucleic acid in the biological sample available to a hybridization reaction.

38. A kit for detecting a mycobacterium in a biological sample comprising:

a) a recombinant BAC vector according to claim 23 or 29, or a purified polynucleotide according to claim 11 ; and

b) reagents necessary to perform a nucleic acid hybridization reaction.

39. A kit for detecting a mycobacterium in a biological sample comprising:

a) a recombinant BAC vector according to claim 23 or 29, or a first polynucleotide according to claim 11 that is immobilized onto a substrate ;

b) reagents necessary to perform a nucleic acid hybridization reaction ; and

c) a second polynucleotide according to claim 11, wherein said second polynucleotide is radioactively or non-radioactively labeled, and wherein said second polynucleotide and said first polynucleotide have non-overlapping sequences.

40. A kit for detecting a mycobacterium in a biological sample comprising:
a) a pair of purified polynucleotides according to claim 20 ; and
b) reagents necessary to perform a nucleic acid amplification reaction.

41. A method for detecting the presence of a genomic DNA, a cDNA or a mRNA of a mycobacterium in a biological sample, comprising the steps of:
a) contacting the biological sample with a plurality of BAC vectors according to claim 23 or 29, or purified polynucleotides according to claim 11 that are immobilized on a substrate ; and
b) detecting the hybrid complexes formed.

42. A kit for detecting a genomic DNA, a cDNA or a mRNA of a mycobacterium in a biological sample, comprising:
a) a substrate on which a plurality of BAC vectors according to claim 23 or 29, or purified polynucleotides according to claim 11 have been immobilized.

43. A method for detecting a polynucleotide of mycobacterial origin in a biological sample, said method comprising:
a) aligning at least one polynucleotide contained in a recombinant BAC vector according to claim 23 or 29 on the surface of a substrate ;
b) contacting the polynucleotide in the biological sample with the substrate on which the polynucleotide of step a) has been aligned ; and
c) detecting a hybrid nucleic acid molecule formed between the polynucleotide in the biological sample and the aligned polynucleotide of step a).

44. A kit for detecting a polynucleotide of mycobacterial origin in a biological sample, comprising:
a) a substrate on which at least one polynucleotide contained in a recombinant BAC vector according to claim 23 or 29 has been aligned.

45. The method of claim 10, wherein the procedure by which the polynucleotide contained in a clone of a BAC DNA library is prepared, further comprises amplifying the polynucleotide insert.

46. The method of claim 10, wherein the procedure by which the polynucleotide contained in a clone of a BAC DNA library is prepared, further comprises digesting the polynucleotide insert with at least one restriction endonuclease.

47. The method of claim 45, further comprising digesting the amplified polynucleotide insert with at least one restriction endonuclease.

48. The Polynucleotide of claim 17, wherein the mycobacterium strain is *Mycobacterium tuberculosis*.

49. The method of claim 36, wherein the amplified mycobacterial DNA is detected by gel electrophoresis or with a labeled polynucleotide according to claim 11.

50. The kit of claim 40, further comprising a polynucleotide according to claim 11.

51. The kit of claim 42, further comprising reagents necessary to perform a hybridization reaction.

52. A method for physically mapping a polynucleotide of mycobacterial origin in a biological sample, said method comprising:

a) aligning at least one polynucleotide contained in a recombinant BAC vector according to claim 23 or 29 on the surface of a substrate;

b) contacting the polynucleotide in the biological sample with the substrate on which the polynucleotide of step a) has been aligned under hybridizing conditions; and

c) detecting the location of the hybridized polynucleotide from the biological sample.

53. The kit of claim 44, further comprising reagents necessary for labeling DNA and reagents necessary for performing a hybridization reaction.